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A POSSIBLE FUNCTION FOR RNA TO DNA
INVERTED TRANSCRIPTION IN ANTIBODY
BIOSYNTHESIS AND IRREVERSIBLE DIFFERENTIATION

by

G. FORTI

1972



Joint Nuclear Research Centre
Ispra Establishment - Italy

Nuclear Study

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ABSTRACT

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A possible function for RNA to DNA inverted transcription
in antibody biosynthesis and irreversible differentiation

Many authors have recently reported that inverted transcription from RNA to DNA was promoted by enzyme present in virions of oncogenic RNA viruses. It was shown that this activity was coded by the viral genes; if however such an activity could be demonstrated as a normal attribute of higher animals, some very interesting possibilities are opening, that are briefly sketched in what follows.

a) Antibody biosynthesis

The sequence determination of Bence-Jones proteins, corresponding to light chains of immunoglobulins, and of heavy chains, have shown that both L and H chains contain invariant (C terminal) and variable (N terminal) sequences. Moreover, indications exist that a single gene is coding for the constant part in each individual, whereas more than one gene is coding for the variable part of the chains (1, 2). These fundamental facts are not explained by the existing theories.

In the primary immune response to antigen, a "priming" of plasmoid cells occurs. These cells begin to synthesize antibodies specific to the given antigen, and become able to elicit secondary response to a further stimulation by the same antigen. In the secondary response, a cell proliferation occurs, and a massive production of antigen takes place in the mature plasma cells.

The primary response has not been obtained in vitro on lymph node cells cultures, however Fischman and others workers (3) have observed such a response when a RNA extract from macrophages incubated with antigen was added to the culture. The first interpretation that mRNA coding for the antibody is transferred to lymphoid cells and is translated there into immunoglobulin chains has been challenged. One of the relevant experiments showed that when macrophages of a rabbit are used to "prime" the lymphocytes of a rabbit of a different allotype, the first antibody produced was of the same allotype as the

macrophages, but after some time the allotype of the antibody reverted to that of the recipient rabbit (4).

Apart from this experiment, it is clear that transfer of mRNA cannot per se solve the problem of the antibody synthesis; it is still not clear how the mRNA, however stabilized, can be transmitted to many daughter cells deriving from the original "primed" cell in secondary reaction, unless we admit that the mRNA is replicated like DNA during cell duplication. The same difficulty arise also if we suppose that the antigen itself, or a fragment of it including the determinant groups is instrumental in the biosynthesis of the antibody in any single plasma cell. It is likely that in secondary reaction there may be more antibody producing cells than there are antigen molecules. If the inverted transcription is postulated, it seems that the following hypothetical scheme offers a break-through in the way to the solution of antibody synthesis problem.

The genes corresponding to the constant parts and many versions of the variable parts of both L and H chains exist separately. In the macrophages both are transcribed and at least the mRNA corresponding to the variable part is translated. These mRNAs are not destroyed but are retained linked to the peptide chains coded by them, in a molecular complex in some specific organelles. An antigen molecule reaching the macrophage selects the variable peptide chains of both the L and H type, with the same chemical specificity governing antigen-antibody reaction. This interaction triggers in the complex a mechanism (of course an enzymatic system must be postulated) that causes the assembling of the mRNA coding for the variable parts with the mRNA coding for the constant part of the respective L and H chains. This requires simply the linkage of the two (or more, see further) mRNAs. The mRNA for the complete L and H chains are now ready. They are released from the organelle and transferred to the recipient plasmoid cell where they are transcribed to DNA and inserted like an episome in the genome of the recipient cell. The insertion in the right place is assured by the fact that the sequence corresponding to the constant part is matching with the base conjugated sequence in the DNA of the recipient cell, and may easily replace the original strand in the double helix, while the variable part results in an extra addition, which may be easily complemented in the template strand by the repair mechanisms of the cell. The plasmoid cell is now "primed" and ready to synthesize antibody in the normal transcription-translation way, as well as to transmit the relevant information to all its progeny. We shall also admit that the new insertion affects the genome in the sense that proliferation is promoted; this will occur (regulation via repression induction mechanism in the first guess) when the primed cell is further stimulated by the same antigen, resulting in secondary response.

It must be noted that reversion after some time to the recipient cell allotype in the experiment quoted is readily understood in this scheme: the allotype marker is in the constant part; the DNA transcribed from mRNA correspond to the transcribed strand of the double helix: a defect of complementarity to the corresponding template strand will be present and after some time will eventually be eliminated by the repair mechanisms of the cell. It is clear that the scheme postulated offers a direct explanation of the existence of constant and variable parts in immunoglobulin chains, as well as of the fact that constant parts are coded for by a single gene, whereas the opposite occurs for variable parts. The scheme offers also a straight-forward explanation of the remarkable phenomenon of allelic exclusion (1), i.e. that each single cell of heterozygous subjects is producing antibody of either one allotype or the other, but not both. Of course "priming" in our scheme, will occur on only one of the homologous chromosomes, and the effect follows immediately. As for what concerns translocation hypothesis in their various form, they offer an explanation for some of the points discussed, however they do not justify the role of transferred RNA in priming; moreover, they face the difficulty of explaining how the DNA sequences corresponding to the variable parts of antibody chains may find the right location adjacent to the proper common sequence. All this difficulties are overcome in our scheme, in which the selection by the antibody, the "priming" of the stem cells, and the relocation of variable sequences near the constant one are all the result of the same integrated process. Additional flexibility in the scheme is given by the fact that we are not bound to maintain that the variable parts are selected as a whole by the antigen. The mRNA assembling process may concern more than two polynucleotides building blocks, thereby reducing further the amount of genetic information necessary to explain the great number of possible antibodies.

This may lessen the strength of the arguments in favour of the somatic random mutability as postulated by clonal selection theory which is rather hard to accept for many people.

b) Irreversible differentiation

In immune response a very peculiar type of cell differentiation occurs.

Let us consider the general problem of irreversible differentiation. It has been shown in many experiments that in higher animals some fundamental steps in differentiation occur, after which the cells, and the clones taking birth from them, are irreversibly committed to a definite path of differentiation. This change must affect the genome itself, as various environmental conditions and even transplantation of nuclei do not invert this differentiation. It is generally accepted that a large part

of genetic information is silent in cells, and different specific genes may be activated in the differentiated cells. However induction-repression mechanism alone cannot explain a real irreversible differentiation transferable to daughter cells.

Even the hystone structure and DNA tridimensional configuration is not likely to cause a true hereditary modification, since both are not expected to survive the dramatic changes in configuration during mitosis, unless one admits that the replication process is not limited to DNA itself, but includes replication of many other molecules. We are thus lead to the assumption that irreversible differentiation must affect the DNA sequence itself.

On the other hand, it is evident that the fundamental differentiation steps must be programmed in some way in the genotype, that is in the DNA of the fertilized egg. We are thus faced with an apparent dilemma.

Some hypothetical mechanism are available for DNA modification based essentially on gene duplication, and subsequent dislocation and recombination. However the problem is to find a mechanism that can produce a specific change in response to a specific signal. A way to solution may be found in a generalization of the scheme proposed for primary immune response. Different DNA sequences may be transcribed, and the corresponding mRNA translated and retained linked to their daughter peptides in a macromolecular complex. A specific protein produced in the same organism, may react with one of the peptides and trigger the fusion of mRNAs. The new mRNA thus assembled may be retranscribed back to DNA and reinserted in a specific location in the same or in a nearby cell.

Such a mechanism will lead to a preprogrammed reshuffling of the genetic information included in the DNA of the genotype, according to specific signals, thus offering a solution to the described dilemma.

c) A new approach to cancer problem?

Myeloma tumor cells which produce Bence-Jones proteins may be regarded as immunocompetent irregularly "primed" cells in which some control mechanism is lost, so that uncontrolled proliferation and constitutive synthesis of immunoglobulin chains develops. It is clear that "priming" (that is, according to our hypothesis, insertion of a new fragment of DNA in a specific position) must affect the genome in the sense of stimulating, under proper conditions, the replication process, as this is the condition for secondary response. In the normal case, the primary response (i.e. the initial differentiation step) leads finally in successive differentiation steps, to plasma cells

which are no more able to proliferate. All this implies specific control mechanism having relation to the extra DNA sequence added. This control may fail in case of myeloma tumor cells. The same situation may apply to the general differentiation scheme: The initial "priming" change is followed by active proliferation and progressive differentiation of a clone, that must be stopped by control mechanism at the right, preprogrammed point.

An irregular "priming" process may lead to the loss of the essential control mechanism because of the specific character of the added DNA sequence and thereby to cancer. This situation parallels very well, in our hypothesis, what is likely to occur in RNA viruses induced tumours. We may admit that such viruses act in the same way as the "priming" mRNA in our hypothesis, and loss of proliferation control results.

We are thus lead to hypothesize the following evolutionistic pathway: A mechanism of irreversible differentiation has been involved in higher animals, based on mRNA reassembling and reversed transcription followed by episomatic reinsertion of DNA, stimulating controlled proliferation. A specialization of this mechanism has led to immunitary response. Cancer may be the consequence of the malfunctioning of these mechanisms.

The author is well aware that all the matter is entirely hypothetical. It seems however that the hypothesis is not unjustified, and may stimulate theoretical discussion and experimental investigation.

Final Note

The ideas expressed in this work were conceived in 1969. The redaction in the present form is of february 1971, stimulated by the publication (Nature, June 1970) of Temin and Baltimore results on the existence of RNA directed DNA polymerase in virions.

Such "reverse transcriptase" enzyme was postulated by the present theory. Now it has been shown that reverse transcription activity exist also in non infected cells, especially in embryos, as it is demanded by the theory. In Temin's laboratory a polynucleotide ligase has been isolated from Rous virions. Temin himself suggests that its function could be that of inserting the DNA resulting from reverse transcription of viral RNA into the host cell genome. All these results bring a strong support the theory; to complete the picture from the point of view of enzymatic activity, a RNA ligase must be found which should weld together the mRNA's in specific situations. A quest for such an enzyme will eventually begin in Ispra in the next months.

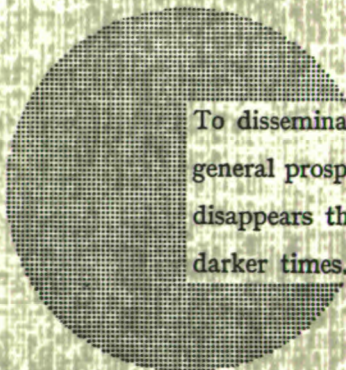
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Alfred Nobel

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